

CLAIMS

1. A method of preparing a multispecific antibody comprising a first polypeptide and at least one additional polypeptide, wherein

(a) the first polypeptide comprises a multimerization domain forming an interface positioned to interact with an interface of a multimerization domain of the additional polypeptide,

(b) the first and additional polypeptides each comprise a binding domain, the binding domain comprising a heavy chain and a light chain, wherein the variable light chains of the first and additional polypeptides comprise a common sequence, the method comprising the steps of:

(I) culturing a host cell comprising nucleic acid encoding the first polypeptide and additional polypeptide, and the variable light chain, wherein the culturing is such that the nucleic acid is expressed; and

(ii) recovering the multispecific antibody from the host cell culture.

2. The method of claim 1, wherein the nucleic acid encoding the first polypeptide or the nucleic acid encoding the additional polypeptide, or both, has been altered from the original nucleic acid to encode the interface or a portion thereof.

3. The method of claim 2 wherein the multimerization domains of one of the first or additional polypeptides, or both, are altered to comprise a free thiol-containing residue which is positioned to interact with a free thiol-containing residue of the interface of the other of the first or additional polypeptide such

that a disulfide bond is formed between the first and additional polypeptides, wherein the nucleic acid encoding the first polypeptide has been altered from the original nucleic acid to encode the free thiol-containing residue or the nucleic acid encoding the additional polypeptide has been altered from the original nucleic acid to encode the free thiol-containing residue, or both.

4. The method of claim 1 wherein the multimerization domains of the first and additional polypeptides comprise a protuberance-into-cavity interaction, wherein the method further comprises:

generating a protuberance by altering the original nucleic acid encoding the first polypeptide to encode an import residue having a larger side chain volume than the original residue, and

generating a cavity by altering the original nucleic acid encoding the additional polypeptide to encode an import residue having a smaller side chain volume than the original residue.

5. The method of claim 4, wherein the steps of generating a protuberance or generating a cavity, or both, occurs by phage display selection.

6. The method of claim 4 wherein the import residue having a larger side chain volume than the original residue is selected from the group consisting of arginine (R), phenylalanine (F), tyrosine (Y), tryptophan (W), isoleucine (I) and leucine (L).

7. The method of claim 4 wherein the import residue having a smaller side chain volume than the original residue is selected from the group consisting of glycine (G), alanine (A), serine (S),

threonine (T), and valine (V), and wherein the import residue is not cysteine (C).

8. The method of claim 1 wherein the first and additional polypeptide each comprise an antibody constant domain.

9. The method of claims 8 wherein the first and additional polypeptide each comprise an antibody constant domain selected from the group consisting of a  $C_H3$  domain and an IgG.

10. The method of claim 1 wherein the heteromultimer is a multispecific immunoadhesin.

11. The method of claim 1 wherein step (I) is preceded by a step wherein the nucleic acid encoding the first and additional polypeptide is introduced into the host cell.

12. A heteromultimer prepared by the method of claim 1.

13. A multispecific antibody comprising a first polypeptide and at least one additional polypeptide which meet at an interface, wherein

(a) the first polypeptide comprises a multimerization domain forming an interface positioned to interact with an interface of a multimerization domain of the additional polypeptide; and

(b) the first and additional polypeptides each comprise a binding domain, the binding domain comprising a variable heavy chain and a variable light chain, wherein the variable light chain of the first and additional polypeptides comprise a common sequence.

14. The multispecific antibody of claim 13, wherein the nucleic acid encoding the first polypeptide or the nucleic acid encoding the additional polypeptide, or both, has been altered from the original nucleic acid to encode the interface or a portion thereof.

15. The multispecific antibody of claim 14 wherein the first polypeptide interface comprises a free thiol-containing residue which is positioned to interact with a free thiol-containing residue of the interface of the additional polypeptide such that a disulfide bond is formed between the first and additional polypeptides, wherein the nucleic acid encoding the first polypeptide has been altered from the original nucleic acid to encode the free thiol-containing residue or the nucleic acid encoding the additional polypeptide has been altered from the original nucleic acid to encode the free thiol-containing residue, or both.

16. The multispecific antibody of claim 14 wherein the interface of the multimerization domains of the first and an additional polypeptide comprise a protuberance and cavity, respectively.

17. The multispecific antibody of claim 16 wherein the protuberance and cavity are generated by alterations in which naturally occurring amino acids are imported into the first and additional polypeptides.

18. A composition comprising the multispecific antibody of claim 13 and a carrier.

19. A host cell comprising nucleic acid encoding the heteromultimer of claim 13.

20. The host cell of claim 19 wherein the host cell is a mammalian cell.

21. A method of preparing a multispecific antibody comprising:

(a) selecting a first nucleic acid encoding a first polypeptide comprising an amino acid residue in the interface of the first polypeptide is replaced with an amino acid residue on an additional polypeptide, and selecting at least one additional nucleic acid encoding at least one additional polypeptide so that the amino acid residue on the additional polypeptide specifically interacts with the amino acid residue on the first polypeptide, thereby generating a stable interaction between the first and additional polypeptides;

(b) selecting a light chain encoding nucleic acid sequence, wherein the light chain is meant to associate with the binding region of each first and additional polypeptide of the multispecific antibody;

(c) introducing into a host cell the first and additional nucleic acids and the light chain-encoding nucleic acid, and culturing the cell so that expression of the first and additional nucleic acids and the light chain-encoding nucleic acid occurs to form the bispecific antibody;

(d) recovering the multispecific antibody from the cell culture.

22. The method of claim 21, wherein at least one of the first and additional nucleic acids of step (a) are altered from the original nucleic acid to encode an amino acid in the interface that interacts with an amino acid of the first or additional amino acid residue thereby generating the stable interaction.

23. The method of claim 22 wherein the altering comprises generating a protuberance-into-cavity interaction at the interface between the first and additional polypeptides.

24. The method of claim 22 wherein the altering comprises importing a free thiol-containing residue into the first or additional polypeptide or both, such that the free thiol-containing residues interact to form a disulfide bond between the first and additional polypeptides.

25. The method of claim 21 wherein the first and additional polypeptide each comprise an antibody constant domain.

26. The method of claim 25 wherein the antibody constant domain is a C<sub>H</sub>3 domain.

27. The method of claim 26 wherein the antibody constant domain is from a human IgG.

28. A method of measuring the formation of a heteromultimeric multispecific antibody comprising a first and at least one additional polypeptide from a mixture of polypeptides, wherein

(a) the first and additional polypeptides meet at an interface of a multimerization domain of each of the first and additional polypeptides,

(b) the interface of the first polypeptide comprises a free thiol-containing residue which is positioned to interact with a free thiol-containing residue of the interface of the additional polypeptide such that a disulfide bond is formed, the method comprising the steps of:

(i) causing each of the multispecific antibodies to migrate in a gel matrix; and

(ii) determining the relative amount of a band corresponding to the multispecific antibody having a non-naturally occurring disulfide bond between the first and additional polypeptides, and a slower migrating band corresponding to a heteromultimer lacking non-naturally occurring disulfide bonds between the first and additional polypeptide.

29. The method of claim 28 wherein the multimerization domains encode a protuberance-into-cavity interaction at the interface, thereby promoting a specific interaction between the first and additional polypeptides.